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DAvenport 1-1200

November 16, 1961

Professor R. K. Morton
Department of Agricultural Chemistry
Waite Agricultural Research Institute
The University of Adelaide
Adelaide, South Australia

Dear Bob:

I feel a report on our progress with the cytochrome b_2 DNA is overdue even though we know less than I thought we would by this time. First let me acknowledge with thanks your last two shipments of the yeast and cytochrome b_2 DNA's. Each arrived frozen and we're impressed by the ingenuity of your packaging.

We have used bacterial alkaline phosphatase (BAP) to determine how much P_i can be released. We wasted time and material learning how to reduce the P_i content of the enzyme, DNA and reagents so that estimates of 1 mμmole or less could be trusted.

Our analysis of the sample received on 10/23 is:

Total organic phosphate	0.93 μmoles/ml.
Deoxypentose	1.05 μmoles/ml. (assuming purines=pyrimidines)
UV	0.99 μmoles/ml. (assuming $\Sigma_{260\ m\mu} = 6.88$)
P_i	18.4 mμmoles/ml.
P_i (after dialysis)	3.5 mμmoles/ml.
P_i released by BAP	10.5 mμmoles/ml. (see enclosed curve)

We are reasonably confident that the BAP has no diesterase since we get theoretical values for pTpT despite large excesses of enzyme and incubation time. We are also encouraged that excess BAP and time in the DNA digestions release no additional P_i. If we assume that all DNA

chains terminated in a monester P and were split, then the average chain contains about 100 residues (0.0105 μmoles P_i/1.0 μmoles nucleotide). We would now like to subject the DNA to the action of splenic diesterase, an exonuclease (Razzell and Khorana, J. Biol. Chem. 236, 1144 (1961); Hilmoe, J. Biol. Chem. 235, 2117 (1960)) which acts only on chains with a free primary hydroxyl group on the terminal nucleoside (i.e., Xp↑Yp↑----). The experiment will be done with and without prior exposure to BAP. Unfortunately our current supply of the splenic enzyme is inadequate for the job and we are working up a fresh batch.

The nearest neighbor analyses were not very revealing. We can see no striking discrepancies in any of the sequences from what is predicted on the basis of random distributions in a DNA of that base composition. What is remarkable is the spread of values for the base compositions of your bulk yeast DNA, the cytochrome b_2 DNA and a sample we prepared from bakers' yeast:

	$\frac{A+T}{G+C}$
Yeast DNA (Morton)	1.65
Yeast DNA (Fleischmann)	2.48
Cytochrome b2	2.14

As you will recognize there is also considerable variation here with your published values. I am also enclosing a copy of a preprint which Mahler sent me which documents these variations in base composition still farther. I'm almost convinced that we must analyze yeast DNA carefully by density gradient centrifugation to determine whether more than one major component is present. What are your ideas?

The Mahler paper is also of interest in the experimental data and conclusion that the DNA is single stranded. Offhand I don't think his data are inconsistent with a double-helical DNA of unusually small size.

So far we've used up only about 2 ml of the 15 ml you sent us. Our contemplated diesterase studies will require very little material. We won't need a lot until we're encouraged to think the DNA is homogeneous and identification of a deoxynucleoside end group is possible. We'll let you know.

We are slightly troubled by a variation between the two samples you sent us in their priming ability for polymerase, the recent one being only 1/3 as active as the first. Here again we'll gain more experience with these samples in due time.

Many thanks again for your enjoyable and stimulating visit. It was fun for all of us.

As ever,

Arthur Kornberg

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